

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION
(PCT Rule 61.2)Date of mailing (day/month/year)
16 November 2000 (16.11.00)To:
Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE
in its capacity as elected OfficeInternational application No.
PCT/US00/06560Applicant's or agent's file reference
INVIT1220WOInternational filing date (day/month/year)
13 March 2000 (13.03.00)Priority date (day/month/year)
19 March 1999 (19.03.99)

Applicant

HEYMAN, John, A. et al

1. The designated Office is hereby notified of its election made: in the demand filed with the International Preliminary Examining Authority on:

23 September 2000 (23.09.00)

 in a notice effecting later election filed with the International Bureau on:2. The election was was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Juan Cruz

Telephone No.: (41-22) 338.83.38

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference INVIT1220WO	FOR FURTHER ACTION		See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US00/06560	International filing date (day/month/year) 13/03/2000	Priority date (day/month/year) 19/03/1999	
International Patent Classification (IPC) or national classification and IPC C12N15/10			
Applicant INVITROGEN CORPORATION			

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 13 sheets, including this cover sheet.

This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I Basis of the report
- II Priority
- III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV Lack of unity of invention
- V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI Certain documents cited
- VII Certain defects in the international application
- VIII Certain observations on the international application

Date of submission of the demand 23/09/2000	Date of completion of this report 25.04.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Page, M Telephone No. +49 89 2399 7322



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/06560

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, pages:

1-17 as originally filed

Claims, No.:

1-34 as originally filed

Drawings, sheets:

1/1 as originally filed

Sequence listing part of the description, pages:

1 (SEQ ID NOs. 1-3), as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- the language of publication of the international application (under Rule 48.3(b)).
- the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- contained in the international application in written form.
- filed together with the international application in computer readable form.
- furnished subsequently to this Authority in written form.
- furnished subsequently to this Authority in computer readable form.
- The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

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the description, pages:

the claims, Nos.:

the drawings, sheets:

5. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):
(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:
 - restricted the claims.
 - paid additional fees.
 - paid additional fees under protest.
 - neither restricted nor paid additional fees.
2. This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is
 - complied with.
 - not complied with for the following reasons:
see separate sheet
4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:
 - all parts.
 - the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)

Yes: Claims 1, 2, 12-15, 19 (partially), 3, 4, 7-11, 16, 32-34 (completely)

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	No:	Claims 1, 2, 12-15, 19 (partially), 5, 6, 17, 18, 20-31 (completely)
Inventive step (IS)	Yes:	Claims 1, 2, 12-15, 19 (partially), 8-11, 32, 33 (completely)
	No:	Claims 1, 2, 12-15, 19 (partially), 3-7, 16-18, 20-31, 34 (completely)
Industrial applicability (IA)	Yes:	Claims 1-34
	No:	Claims

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/US00/06560

The application concerns the provision of a method for isolating and cloning full-length cDNA sequences. Messenger RNA is first reverse transcribed and the cDNA/mRNA hybrids are then isolated via the mRNA cap structure (optionally after removal of unhybridised RNA), the 3' end of the first-strand DNA is 'tagged' and second strand synthesis is performed using the sequence of the tag as a binding site for specially designed primers.

Re Item IV

Lack of Unity of Invention

An international application must relate to one invention only or to a group of inventions so linked as to form a single general inventive concept. Unity of invention is fulfilled only when there is a technical relationship between the inventions involving one or more of the same or corresponding special technical features. Special technical features are such features that define the contribution of the claimed invention over the prior art.

The identified 6 inventions relate to methods of isolation of full-length double-stranded cDNA which involves the technical features of claim 1 (first-strand cDNA synthesis from mRNA, denaturation of hybrid products, attachment of a tag sequence to the 3' end of the first strand cDNA and production of the second strand using the first tagged strand) as the sole common link. However, this feature cannot be considered to constitute a special technical feature because it does not define a contribution over the prior art. This method is already known in the prior art (D1 Fig. 1 and Materials and Methods pages 328-329). Because the methods of claims 3, 5, 7, 8, 10, 12 and 14 are all separately dependent on either claim 1 or claim 2, there is no special technical feature linking these claims and their dependent subject matter.

Furthermore, the IPEA considers that there is no technical relationship between the subject matter of claims 1-33 (methods for cloning full-length cDNA sequences and associated products) and claim 34 (a fusion protein comprising eIF4E and eIF4G). Because the isolation of full-length cDNA according to the method of claims 1-33 is not restricted to the use of an eIF4E:eIF4G fusion protein and because products for the isolation of full-length mRNA via the 5' cap are known (D2 column 5 line 30),

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these two groups of claims are considered to solve two different technical problems and claim 34 therefore constitutes a further invention.

The inventions are considered to be defined as follows:

Invention I Claims 1, 2, 12-15, 19-31 (all partially), 3, 4 and 16 (completely)

Methods for the isolation of full-length double-stranded cDNA comprising first-strand cDNA synthesis from mRNA, denaturation of hybrid products, attachment of a tag sequence to the 3' end of the first strand cDNA and production of the second strand using the first tagged strand, whereby the mRNA is isolated via cap-binding proteins.

Invention II Claims 1, 2, 12-15, 19-31 (all partially), 5, 6, 17 and 18 (completely)

Methods for the isolation of full-length double-stranded cDNA comprising first-strand cDNA synthesis from mRNA, denaturation of hybrid products, attachment of a tag sequence to the 3' end of the first strand cDNA and production of the second strand using the first tagged strand, whereby the mRNA is isolated via biotinylation of the cap structure and subsequent affinity purification.

Invention III Claims 1, 2, 12-15, 19-31 (all partially) and 7 (completely)

Methods for the isolation of full-length double-stranded cDNA comprising first-strand cDNA synthesis from mRNA, denaturation of hybrid products, attachment of a tag sequence to the 3' end of the first strand cDNA and production of the second strand using the first tagged strand, whereby the mRNA is de-capped and de-phosphorylated subsequent to isolation.

Invention IV Claims 1, 2, 12-15, 19-31 (all partially), 8 and 9 (completely)

Methods for the isolation of full-length double-stranded cDNA comprising first-strand cDNA synthesis from mRNA, denaturation of hybrid products, attachment of a tag sequence to the 3' end of the first strand cDNA and production of the second strand using the first tagged strand, whereby the tag comprises recognition sites for recombinases and/or restriction endonucleases.

**Invention V Claims 1, 2, 12-15, 19-31 (all partially), 10, 11, 32 and 33
(completely)**

Methods for the isolation of full-length double-stranded cDNA comprising first-strand cDNA synthesis from mRNA, denaturation of hybrid products, attachment of a tag sequence to the 3' end of the first strand cDNA and production of the second strand using the first tagged strand, whereby the tag is attached to the first strand cDNA via a site-specific recombinase capable of recognising and acting on single-stranded DNA.

Invention VI Claim 34

A fusion protein comprising eIF4E and eIF4G.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1) Reference is made to the following documents:

- D1: CARNINCI P ET AL: 'High-efficiency full-length cDNA cloning by biotinylated CAP trapper' GENOMICS, US, ACADEMIC PRESS, SAN DIEGO, vol. 37, no. 3, 1 November 1996 (1996-11-01), pages 327-336, XP002081729 ISSN: 0888-7543
- D2: EP-A-0 373 914 (UNIV MCGILL) 20 June 1990 (1990-06-20)
- D3: EDERY I ET AL: 'AN EFFICIENT STRATEGY TO ISOLATE FULL-LENGTH CDNAS BASED ON AN mRNA CAP RETENTION PROCEDURE (CAPTURE)' MOLECULAR AND CELLULAR BIOLOGY, US, WASHINGTON, DC, vol. 15, June 1995 (1995-06), pages 3363-3371, XP002915963 ISSN: 0270-7306 cited in the application
- D4: WO 98 56943 A (SLOAN KETTERING INST CANCER ;INVITROGEN CORP (US)) 17 December 1998 (1998-12-17)
- D5: WO 97 24455 A (CLONTECH LAB INC) 10 July 1997 (1997-07-10)
- D6: EP-A-0 625 572 (KATO SEISHI ;SEKINE SHINGO (JP); KANAGAWA KAGAKU GIJUTSU AKAD (JP)) 23 November 1994 (1994-11-23)
- D7: A. HAGHIGHAT AND N. SONENBERG: 'eIF4G dramatically enhances the binding of eIF4E to the mRNA 5'-Cap structure' J. BIOL. CHEM., vol. 272, no.

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International application No. PCT/US00/06560

35, 29 August 1997 (1997-08-29), pages 21677-21680, XP002141735 AM.
SOC. BIOCHEM. MOL.BIOL.,INC.,BALTIMORE,US cited in the application

D8: A. HAGHIGHAT ET AL.: 'The eIF4G-eIF4E complex is the target for direct cleavage by the rhinovirus 2A proteinase' J. VIROLOGY, vol. 70, no. 12, December 1996 (1996-12), pages 8444-8450, XP002141736 AM.SOC.MICROBIOL.,WASHINGTON,US

D9: R J DIGATE ET AL: 'Molecular Cloning and DNA Sequence Analysis of Escherichia coli topB, the gene encoding Topoisomerase III' JOURNAL OF BIOLOGICAL CHEMISTRY,US,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 264, no. 30, 25 October 1989 (1989-10-25), pages 17924-17930-17930, XP002122019 ISSN: 0021-9258

2) **Novelty - Art.33(1) and (2) PCT:**

Invention I

Claims 1, 2, 12-15, 19 (all partially), 3, 4 and 16 (completely) appear to be novel in light of the cited prior art, which nowhere combines the use of cap-binding proteins and the attachment of a non-native tag sequence to the first stand cDNA.

Invention II

Claims 1, 2, 12-15, 19 (all partially), 5, 6, 17 and 18 (completely) lack novelty in light of D1, which discloses a method for isolating full-length cDNA in Fig. 1, whereby the cap structure of mRNA is biotinylated, first strand cDNA is synthesised via reverse transcription, un-hybridised RNA is removed through digestion with RNase I, cDNA is tagged with oligo dG, the product is isolated via streptavidin beads, the mRNA removed and second strand synthesis carried out using the oligo G tag. See also D1 pages 328 and 329 Materials and Methods. The sequences are cloned into pBluescript via Lambda Zap II.

Invention III

Claims 1, 2, 12-15, 19 (all partially) and 7 (completely) appear to be novel in light of the cited prior art, which nowhere combines the attachment of a non-native tag sequence to the first stand cDNA with a method whereby the mRNA is de-capped and de-phosphorylated after isolation.

Invention IV

Claims 1, 2, 12-15, 19 (all partially), 8 and 9 (completely) appear to be novel in light of the cited prior art, which nowhere discloses the attachment of a non-native tag sequence comprising recognition sites for recombinases and/or restriction endonucleases to the first stand cDNA.

Invention V

Claims 1, 2, 12-15, 19 (all partially), 10, 11, 32 and 33 (completely) appear to be novel in light of the cited prior art, which nowhere discloses the attachment of a non-native tag sequence to the first stand cDNA via a site-specific recombinase capable of recognising and acting on single-stranded DNA.

Inventions I-V

Claims 20-31

A previously disclosed compound is not rendered novel by a method for its production. The subject matter of **claim 20** and therefore also of **claims 21-31** is indistinguishable from full-length sequences isolated using different protocols and can therefore not be acknowledged as being novel.

Invention VI

The fusion protein of **claim 34** does not appear to have been previously disclosed in the cited prior art and is therefore considered to be novel.

3) **Inventive Step - Art.33(1) and (3) PCT:**

The following comments on inventive step are confined to subject matter which could be acknowledged as being novel, or for which novelty could potentially be restored as outlined supra.

Invention I Claims 1, 2, 12-15, 19 (all partially), 3, 4 and 16 (completely)

The closest prior art is document D1, which discloses a method for the isolation of full-length double-stranded cDNA sequences through the isolation of mRNA via the 5' cap, the addition of an oligo dG tag to the 3' end of the resulting first strand cDNA and the synthesis of a second strand using the tag as a primer target.

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In light of the prior art, the technical problem can be regarded as the provision of an alternative method for the isolation of mRNA for the purposes of synthesising full-length double-stranded cDNA.

The technical problem is solved by the subject matter of **claims 1, 2, 12-15, 19 (all partially), 3, 4 and 16 (completely)** which provide methods for the isolation of mRNA through the use of cap-binding proteins.

To one skilled in the art the solution provided by **claims 1, 2, 12-15, 19 (all partially), 3, 4 and 16 (completely)** would be obvious. D2 discloses the use of eIF-4E in capturing mRNA for full-length double-stranded cDNA synthesis (D2 column 5 line 30). Inventive step cannot be acknowledged for Invention I.

Invention III Claims 1, 2, 12-15, 19 (all partially) and 7 (completely)

Claim 7 lacks support in the description and it is not possible to determine which technical problem is solved by the said claim. It is therefore not possible to acknowledge inventive step for **claims 1, 2, 12-15, 19 (all partially) and 7 (completely)**.

Invention IV Claims 1, 2, 12-15, 19 (all partially), 8 and 9 (completely)

The closest prior art is document D1, which discloses a method for the isolation of full-length double-stranded cDNA sequences through the isolation of mRNA via the 5' cap, the addition of an oligo dG tag to the 3' end of the resulting first strand cDNA and the synthesis of a second strand using the tag as a primer target.

In light of the prior art, the technical problem can be regarded as the provision of an alternative method for the synthesis of full-length double-stranded cDNA from mRNA, whereby sites useful for the subsequent cloning of the cDNA are introduced.

The technical problem is solved by the subject matter of claims 1, 2, 12-15, 19 (all partially), 8 and 9 (completely) which provide tag sequences comprising recombinase or restriction endonuclease recognition sites.

The solution provided by **claims 1, 2, 12-15, 19 (all partially), 8 and 9 (completely)** appears to be inventive in light of the cited prior art. Although D4 discloses the

incorporation of topoisomerase and restriction endonuclease sequences into the mRNA via Vaccinia topoisomerase during the isolation of full-length gene sequences (D4 claims 45, 58 and 59), there does not appear to be any motivation in D1 or D4 to carry out a similar procedure for first strand cDNA.

Invention V Claims 1, 2, 12-15, 19 (all partially), 10, 11, 32 and 33 (completely)

The closest prior art is again document D1, which discloses a method for the isolation of full-length double-stranded cDNA sequences through the isolation of mRNA via the 5' cap, the addition of an oligo dG tag to the 3' end of the resulting first strand cDNA via terminal deoxynucleotidyl transferase and the synthesis of a second strand using the tag as a primer target.

In light of the prior art, the technical problem can be regarded as the provision of an alternative enzyme for adding tags to the 3' end of first strand cDNA in order to facilitate the cloning of full-length double-stranded cDNA from mRNA.

The technical problem is solved by the subject matter of claims 1, 2, 12-15, 19 (all partially), 10, 11, 32 and 33 (completely) which provide a method for isolating full-length double-stranded cDNA whereby a tag is attached to the first strand cDNA via a site-specific recombinase capable of recognising and acting on single-stranded DNA.

The solution provided by **claims 1, 2, 12-15, 19 (all partially), 10, 11, 32 and 33 (completely)** appears to be inventive in light of the cited prior art. D1 uses a terminal transferase in order to add a tag to the 3' end of the first strand cDNA. However, this enzyme is only capable of introducing bases to the cDNA molecule one by one, in contrast to the recombinases of the application which can add selected sequences to the said molecule. Although D4 discloses the use of topoisomerase to ligate DNA sequences to the mRNA molecule after removal of the cap, there does not appear to be any motivation in the prior art to use such an enzyme to add these sequences to the first cDNA strand and thus avoiding the necessity to de-cap and dephosphorylate the mRNA.

Invention VI Claim 34

The closest prior art is D2, which provides protein A/eIF4E fusion proteins for the isolation of full-length mRNA.

In light of the prior art, the technical problem can be regarded as the provision of an alternative eIF4E fusion protein.

The provision of eIF4E / eIF4G fusion proteins is considered to be trivial and not to contribute to the teaching of the art. In the absence of any functional statement in claim 34, it cannot be seen to solve any technical problem and it is therefore not possible to acknowledge inventive step.

N.B. Even if a purposive statement in line with the description were to be added to claim 34, it would still not possible to acknowledge inventive step for the said claim. No concrete examples are given of functional eIF4E / eIF4G cap-binding fusion proteins. It is considered that not every eIF4E / eIF4G fusion protein will function as a cap-binding protein and thus the technical problem is not solved by all the claimed subject matter.

Re Item VII

Certain defects in the international application

- a) Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1, D2, D4-D6, D8 and D9 are not mentioned in the description, nor are these documents identified therein.
- b) The double citation of eIF4G in claim 4 leaves the reader in doubt as to what subject matter protection is sought for.

Re Item VIII

Certain observations on the international application

- a) The term "incorporated by reference", e.g. on page 3 lines 25-26, page 8 lines 9 and 10, should be removed. A patent application must be self understanding; the objected

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International application No. PCT/US00/06560

term renders the scope of the application obscure (Art. 5 and 6, Rule 9.1(iv) PCT).

- b) It is clear from the description on page 7 and the examples on pages 15-17 that the use of type I topoisomerases is essential to the definition of the invention. Since independent claim 1 does not contain this feature, it does not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.
- c) The term "non-native tag sequence" in claim 1 is unclear and renders the scope of the said claim obscure (Article 6 PCT).
- d) Claim 7 lacks support from the description. Nowhere in the description is there any reference to the de-capping or de-phosphorylation of the mRNA. The said claim should be brought into line with the description (Art. 6 PCT). The applicant is reminded that in correcting this deficiency it is not permissible for subject matter to be added.
- e) It is not explicitly clear in claim 3 which step the affinity purification is to be applied to (Article 6 PCT).
- f) Claim 10 lacks essential technical features. It is clear from the description that the method has only been carried out with *E. coli* topoisomerase III (page 7 lines 15- 30) (Articles 5 and 6 PCT).
- g) Although the terms "pYES2", "pMT", "pIND", and "pcDNA3.1" are known in the art, it is not clear exactly what the sequences being referred to are. The terms should be qualified, e.g. through reference to a genebank accession number or equivalent. Such products are not constant over time and the meaning of the terms therefore lacks the clarity required by Article 6 PCT.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

102894 - 159943

<p>To:</p> <p>Gray Cary Ware & Freidenrich LLP Attn. Reiter, Stephen E. 4365 Executive Drive, Suite 1600 San Diego, CA 92121-2189 UNITED STATES OF AMERICA</p>	<p style="margin: 0;">Date of mailing (day/month/year)</p> <p style="margin: 0;">17/07/2000</p>
<p>Applicant's or agent's file reference</p> <p>INVIT1220WO</p>	<p>FOR FURTHER ACTION See paragraphs 1 and 4 below</p>
<p>International application No.</p> <p>PCT/US 00/06560</p>	<p>International filing date (day/month/year)</p> <p>13/03/2000</p>
<p>Applicant</p> <p>INVITROGEN CORPORATION</p>	

1. The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90bis.1 and 90bis.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

<p>Name and mailing address of the International Searching Authority</p> <p>European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016</p>	<p>Authorized officer</p> <p>Mireille Claudepierre</p>
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PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference INVIT1220WO	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/US 00/06560	International filing date (day/month/year) 13/03/2000	(Earliest) Priority Date (day/month/year) 19/03/1999
Applicant INVITROGEN CORPORATION		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
 - the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :
 - contained in the international application in written form.
 - filed together with the international application in computer readable form.
 - furnished subsequently to this Authority in written form.
 - furnished subsequently to this Authority in computer readable form.
 - the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
 - the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished
- 2. **Certain claims were found unsearchable** (See Box I).
- 3. **Unity of invention is lacking** (see Box II).

4. With regard to the title,

- the text is approved as submitted by the applicant.
- the text has been established by this Authority to read as follows:

5. With regard to the abstract,

- the text is approved as submitted by the applicant.
- the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

- as suggested by the applicant.
- because the applicant failed to suggest a figure.
- because this figure better characterizes the invention.

1

None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/06560

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/10 C12N15/62 C12N15/70 C12N15/85 C12N9/90
C07K14/47 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12Q C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, PAJ, CAB Data, STRAND, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CARNINCI P ET AL: "High-efficiency full-length cDNA cloning by biotinylated CAP trapper" GENOMICS, US, ACADEMIC PRESS, SAN DIEGO, vol. 37, no. 3, 1 November 1996 (1996-11-01), pages 327-336, XP002081729 ISSN: 0888-7543 the whole document	1,2, 14-21
Y	---	3,4, 22-31

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

4 July 2000

17/07/2000

Name and mailing address of the ISA

Europena Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Hornig, H

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/06560

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	P. CARNINCI ET AL.: "High efficiency selection of full-length cDNA by improved biotinylated cap trapper" DNA RESEARCH, vol. 4, 1997, pages 61-66, XP002915968 KAZUSA DNA RES. UNIV.ACAD.PRESS, INC., TOKYO, JP cited in the application the whole document ---	1,2, 14-21
Y		3,4, 22-31
X	EP 0 373 914 A (UNIV MCGILL) 20 June 1990 (1990-06-20) the whole document ---	20,21
Y		3,4, 22-31
X	EDERY I ET AL: "AN EFFICIENT STRATEGY TO ISOLATE FULL-LENGTH CDNAS BASED ON AN mRNA CAP RETENTION PROCEDURE (CAPTURE)" MOLECULAR AND CELLULAR BIOLOGY, US, WASHINGTON, DC, vol. 15, June 1995 (1995-06), pages 3363-3371, XP002915963 ISSN: 0270-7306 cited in the application the whole document ---	20,21
Y		3,4, 22-31
X	WO 98 56943 A (SLOAN KETTERING INST CANCER ; INVITROGEN CORP (US)) 17 December 1998 (1998-12-17) claims 1-79; figure 11A ---	20,21
X	WO 97 24455 A (CLONTECH LAB INC) 10 July 1997 (1997-07-10) the whole document ---	20,21
X	WO 98 20122 A (HAYASHIZAKI YOSHIHIDE ; INST OF PHYSICAL AND CHEMICAL (JP)) 14 May 1998 (1998-05-14) the whole document ---	20,21
X	WO 98 55502 A (SMITHKLINE BEECHAM CORP ; BERGSMA DERK J (US); MOONEY JEFFREY L (US) 10 December 1998 (1998-12-10) the whole document ---	20,21
X	KATO S ET AL: "CONSTRUCTION OF A HUMAN FULL-LENGTH CDNA BANK" GENE, NL, ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, vol. 150, 1994, pages 243-250, XP002915961 ISSN: 0378-1119 the whole document ---	20,21
	-/-	

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/06560

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MARUYAMA K ET AL: "OLIGO-CAPPING: A SIMPLE METHOD TO REPLACE THE CAP STRUCTURE OF EUKARYOTIC MRNAs WITH OLIGONUCLEOTIDES" GENE, NL, ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, vol. 138, 1 January 1994 (1994-01-01), pages 171-174, XP002017391 ISSN: 0378-1119 the whole document ----	20, 21
X	EP 0 625 572 A (KATO SEISHI ;SEKINE SHINGO (JP); KANAGAWA KAGAKU GIJUTSU AKAD (JP)) 23 November 1994 (1994-11-23) the whole document ----	20, 21
X	WO 96 34981 A (GENSET ;DUMAS MILNE EDWARDS JEAN BAPTISTE (FR); MERENKOVA IRENA NICOL) 7 November 1996 (1996-11-07) the whole document ----	20, 21
Y	INVITROGEN PRODUCT KATALOG 1998, page 18,29,43,44,49-52 XP002141734 Carlsbad, CA, US pYES2, pMT, pIND or pCDNA3.1 ----	22-31
A	A. HAGHIGHAT AND N. SONENBERG: "eIF4G dramatically enhances the binding of eIF4E to the mRNA 5'-Cap structure" J. BIOL. CHEM., vol. 272, no. 35, 29 August 1997 (1997-08-29), pages 21677-21680, XP002141735 AM. SOC. BIOCHEM. MOL.BIOL., INC., BALTIMORE, US cited in the application the whole document ----	
A	A. HAGHIGHAT ET AL.: "The eIF4G-eIF4E complex is the target for direct cleavage by the rhinovirus 2A proteinase" J. VIROLOGY, vol. 70, no. 12, December 1996 (1996-12), pages 8444-8450, XP002141736 AM. SOC. MICROBIOL., WASHINGTON, US the whole document ----	
		-/-

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/06560

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>R J DIGATE ET AL: "Molecular Cloning and DNA Sequence Analysis of <i>Escherichia coli</i> <i>topB</i>, the gene encoding Topoisomerase III" <i>JOURNAL OF BIOLOGICAL CHEMISTRY</i>, US, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 264, no. 30, 25 October 1989 (1989-10-25), pages 17924-17930-17930, XP002122019 ISSN: 0021-9258 the whole document</p> <p>---</p>	
A	<p>WO 96 19497 A (SLOAN KETTERING INST CANCER) 27 June 1996 (1996-06-27) the whole document</p> <p>-----</p>	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/06560

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP 0373914	A 20-06-1990	CA 2005252 A		13-06-1990
		DE 68916769 D		18-08-1994
		DE 68916769 T		10-11-1994
		US 5219989 A		15-06-1993
WO 9856943	A 17-12-1998	AU 8256598 A		30-12-1998
		EP 0920526 A		09-06-1999
WO 9724455	A 10-07-1997	US 5962271 A		05-10-1999
		EP 0871780 A		21-10-1998
		JP 2000502905 T		14-03-2000
		US 5962272 A		05-10-1999
WO 9820122	A 14-05-1998	JP 10127291 A		19-05-1998
		EP 0990702 A		05-04-2000
WO 9855502	A 10-12-1998	EP 0988313 A		29-03-2000
EP 0625572	A 23-11-1994	JP 6153953 A		03-06-1994
		WO 9408001 A		14-04-1994
		US 5597713 A		28-01-1997
WO 9634981	A 07-11-1996	FR 2733765 A		08-11-1996
		FR 2733762 A		08-11-1996
		AU 719217 B		04-05-2000
		AU 5982996 A		21-11-1996
		CA 2220045 A		07-11-1996
		EP 0824598 A		25-02-1998
		JP 11510364 T		14-09-1999
		US 6022715 A		08-02-2000
WO 9619497	A 27-06-1996	US 5766891 A		16-06-1998
		AU 705983 B		03-06-1999
		AU 4515096 A		10-07-1996
		CA 2208062 A		27-06-1996
		EP 0828755 A		18-03-1998
		JP 10510716 T		20-10-1998

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

- (i) the claim is unchanged;
- (ii) the claim is cancelled;
- (iii) the claim is new;
- (iv) the claim replaces one or more claims as filed;
- (v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

RECEIVED

JUL 23 2001

GRAYCARY/J.VAIL

From the INTERNATIONAL SEARCHING AUTHORITY

To:
 LISA HAILE
 GRAY CARY WARE & FREIDENRICH
 4365 EXECUTIVE DRIVE, SUITE 1600
 SAN DIEGO, CA 92121-2189

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT
OR THE DECLARATION

(PCT Rule 44.1)

		Date of Mailing (day/month/year)
Applicant's or agent's file reference INVIT1270WO		FOR FURTHER ACTION See paragraphs 1 and 4 below
International application No. PCT/US01/05745		International filing date (day/month/year) 23 February 2001 (23.02.2001)
Applicant INVITROGEN CORPORATION		

1. The applicant is hereby notified that the international search report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the international search report; however, for more details, see the notes on the accompany sheet.

Where? Directly to the International Bureau of WIPO

34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

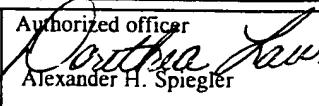
Shortly after 18 months from the priority date, the international application will be published by the International Bureau.

If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in rules 90 bis 1 and 90 bis 3, respectively, before the completion of the technical preparations for international publication.

Within 19 months from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within 20 months from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the ISA/US
 Commissioner of Patents and Trademarks
 Box PCT
 Washington, D.C. 20231
 Facsimile No. (703)305-3230

Authorized officer

 Alexander H. Spiegler
 Telephone No. (703) 308-0196

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:
 LISA HAILE
 GRAY CARY WARE & FREIDENRICH
 4365 EXECUTIVE DRIVE, SUITE 1600
 SAN DIEGO, CA 92121-2189

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT
OR THE DECLARATION

(PCT Rule 44.1)

		Date of Mailing (day/month/year) 17 JUL 2001
Applicant's or agent's file reference INVIT1270WO		FOR FURTHER ACTION See paragraphs 1 and 4 below
International application No. PCT/US01/05745		International filing date (day/month/year) 23 February 2001 (23.02.2001)
Applicant INVITROGEN CORPORATION		

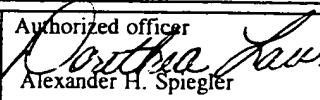
1. The applicant is hereby notified that the international search report has been established and is transmitted herewith.
Filing of amendments and statement under Article 19:
 The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):
When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the international search report; however, for more details, see the notes on the accompanying sheet.
Where? Directly to the International Bureau of WIPO
 34, chemin des Colombettes
 1211 Geneva 20, Switzerland
 Facsimile No.: (41-22) 740.14.35
 For more detailed instructions, see the notes on the accompanying sheet.

2. The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:
 the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.
 no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:
 Shortly after 18 months from the priority date, the international application will be published by the International Bureau.
 If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in rules 90 bis 1 and 90 bis 3, respectively, before the completion of the technical preparations for international publication.
 Within 19 months from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).
 Within 20 months from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the ISA/US
 Commissioner of Patents and Trademarks
 Box PCT
 Washington, D.C. 20231
 Facsimile No. (703)305-3230

Authorized officer

 Alexander H. Spiegler
 Telephone No. (703) 308-0196

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference INVIT1270WO	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/US01/05745	International filing date (day/month/year) 23 February 2000 (23.02.2000)	(Earliest) Priority Date (day/month/year) 25 February 2000 (25.02.2000)
Applicant INVITROGEN CORPORATION		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.



It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the Report**
 - a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
 - the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
 - b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence listing:
 - contained in the international application in written form.
 - filed together with the international application in computer readable form.
 - furnished subsequently to this Authority in written form.
 - furnished subsequently to this Authority in computer readable form.
 - the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
 - the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.
2. Certain claims were found unsearchable (See Box I).
3. Unity of invention is lacking (See Box II).
4. With regard to the title,
 - the text is approved as submitted by the applicant.
 - the text has been established by this Authority to read as follows:
5. With regard to the abstract,
 - the text is approved as submitted by the applicant.
 - the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.
6. The figure of the drawings to be published with the abstract is Figure No.
 - as suggested by the applicant.
 - because the applicant failed to suggest a figure.
 - because this figure better characterizes the invention.

None of the figures

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/05745

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 15/64, C12P 19/34, C12N 15/00
 US CL : 435/91.41, 91.1, 91.4, 91.5, 172.3

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/91.41, 91.1, 91.4, 91.5, 172.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,766,891 A (SHUMAN) 16 June 1998 (16.06.1998), see whole document.	1-3 and 6
A,P	US 6,238,884 B1 (SHORT et al) 29 May 2001 (29.03.2001), see whole document.	1-39

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

15 June 2001 (15.06.2001)

Date of mailing of the international search report

17 JUL 2001

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks
 Box PCT
 Washington, D.C. 20231

Facsimile No. (703)305-3230

Authorized officer

Alexander H. Spiegler
Alexander H. Spiegler

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/05745

Continuation of B. FIELDS SEARCHED Item3:

Databases: WEST, Medline, Biosis, CAPlus, Embase, Biotechds, Sequence Search

Search Terms: topoisomerase, linker, PCR, biotin, Vaccinia, Taq polymerase, overhang, sequencing

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under Article 19. The Notes are based on the requirements of the Patent Cooperation Treaty and of the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule" and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

The claims only.

The description and the drawings may only be amended during international preliminary examination under Chapter II.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/ is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confounded with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

- (i) the claim is unchanged;
- (ii) the claim is cancelled;
- (iii) the claim is new;
- (iv) the claim replaces one or more claims as filed;
- (v) the claim is the result of the division of a claim as filed.

NOTES TO FORM PCT/ISA/220 (continued)

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under Article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

The statement should be brief, it should not exceed 500 words if in English or if translated into English.

It should not be confounded with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It should not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

In what language?

The amendments must be made in the language in which the international application is published. The letter and any statement accompanying the amendments must be in the same language as the international application if that language is English or French; otherwise, it must be in English or French, at the choice of the applicant.

Consequence if a demand for international preliminary examination has already been filed?

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase?

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.